

Endonuclease from gram-negative bacteria *Serratia marcescens* is as effective as pulmozyme in the hydrolysis of DNA in sputum

Vafina G., Zainutdinova E., Bulatov E., Filimonova M.
Kazan Federal University, 420008, Kremlevskaya 18, Kazan, Russia

Abstract

© 2018 Vafina, Zainutdinova, Bulatov and Filimonova. One of the approaches to effective airway cleansing is the degradation of DNA into smaller fragments. For this purpose Pulmozyme® is used with high efficacy because it contains recombinant DNase I as its active component. The aim of the study was to comparatively analyze DNase activity of Pulmozyme® and the nuclease from gram-negative bacteria *Serratia marcescens*, because at optimal conditions the catalytic efficiency of the nuclease is much higher than the efficiency of DNase I. Highly polymerized DNA and purulent-mucous sputum were used as substrates. The examination showed that both *S. marcescens* nuclease and Pulmozyme® hydrolyzed DNA in sputum. Also *S. marcescens* nuclease was found capable of hydrolyzing DNA in conditions that are standard for Pulmozyme® and suitable for its therapeutic application. For manifesting the similar hydrolytic activity the nuclease amount in the assay mixture containing highly polymerized DNA or the sonicated sputum and NaCl together with calcium- or magnesium- cations can be about 10- time lower than that of the recombinant DNase I. In the presence of magnesium cations the DNase activity of both *S. marcescens* nuclease and Pulmozyme® was higher than in the presence of calcium cations.

<http://dx.doi.org/10.3389/fphar.2018.00114>

Keywords

Hydrolysis of DNA, Pulmozyme, *Serratia marcescens* nuclease, Sma nuc, Sputum

References

- [1] Ball, T., Wasmuth, C., Braunagel, C., and Benedik, M. (1990). Expression of *Serratia marcescens* extracellular proteins requires recA. *J. Bacteriol.* 172, 324-349. doi: 10.1128/jb.172.1.342-349.1990
- [2] Benedik, M., and Strych, U. (1998). *Serratia marcescens* and its extracellular nuclease. *FEMS Microbiol. Lett.* 165, 1-13. doi: 10.1111/j.1574-6968.1998.tb13120.x
- [3] Berkmen, M., and Benedik, M. (2002). Multi-Copy repression of *Serratia marcescens* nuclease expression by *dini*. *Curr. Microbiol.* 44, 44-48. doi: 10.1007/s00284-001-0072-y
- [4] Biedermann, K., Jepsen, P., Riise, E., and Svendsen, I. (1989). Purification and characterization of a *Serratia marcescens* nuclease produced by *Escherichia coli*. *Carlsberg Res. Commun.* 54, 17-27. doi: 10.1007/BF02910469
- [5] Chen, C., Krause, K., and Pettitt, B. (2009). Advantage of being a dimer for *Serratia marcescens* endonuclease. *J. Phys. Chem. B* 113, 511-521. doi: 10.1021/jp8057838

- [6] Filimonova, M., Garusov, A., Smetanina, T., Andreeva, M., Bogomol'naya, L., and Leshchinskaya, I. (1996). Isoforms of *Serratia marcescens* nuclease. comparative analysis of the substrate specificity. *Biokhimiya* 61, 1800-1806
- [7] Filimonova, M., Gubskaya, V., and Nuretdinov, I. (2014). Some features of hydrolysis of the hybrid B-Z-form DNA by *Serratia marcescens* nuclease. *OnLine J. Biol. Sci.* 14, 179-185. doi: 10.3844/ojbsci.2014.181.187
- [8] Filimonova, M., Gubskaya, V., Nuretdinov, I., Benedik, M., Bogomol'naya, L., Andreeva, M., et al. (1997). Isoforms of *Serratia marcescens* nuclease. The role of Mg^{2+} in the hydrolysis mechanism. *Biochemistry* 62, 1148-1154
- [9] Filimonova, M., Gubskaya, V., Nuretdinov, I., Benedik, M., Cherepanova, N., and Leshchinskaya, I. (2001). Study of the mechanism of action of p-chloromercuribenzoate on endonuclease from the bacterium *Serratia marcescens*. *Biochemistry* 66, 323-327
- [10] Filimonova, M., Gubskaya, V., Nuretdinov, I., and Leshchinskaya, I. (2003). Action of hexaamincobalt on the activity of *Serratia marcescens* nuclease. *Biometals* 16, 447-453. doi: 10.1023/A:1022583929659
- [11] Friedhoff, P., Kolmes, B., Gimadutdinov, O., Wende, W., Krause, K., and Pingoud, A. (1996a). Analysis of the mechanism of the *Serratia* nuclease using site-directed mutagenesis. *Nucleic Acids Res.* 24, 2632-2639
- [12] Friedhoff, P., Meiss, G., Kolmes, B., Pieper, U., Gimadutdinov, O., Urbanke, C., et al. (1996b). Kinetic analysis of the cleavage of natural and synthetic substrates by the *Serratia* nuclease. *Eur. J. Biochem.* 241, 572-580
- [13] Gullberg, U., Andersson, E., Garwicz, D., Lindmark, A., and Olsson, I. (1997). Biosynthesis, processing and sorting of neutrophil proteins: insight into neutrophil granule development. *Eur. J. Haematol.* 58, 137-153. doi: 10.1111/j.1600-0609.1997.tb00940.x
- [14] Johnson, J. (1994). "Similarity analysis of DNAs". in *Methods for General and Molecular Bacteriology*", ed. P. Gerhardt (Washington, DC: American Society for Microbiology), 655-682
- [15] Kalckar, H. (1947). Differential spectrophotometry of purine compounds by means of specific enzymes. I. Determination of hydroxypurine compounds. *J. Biol. Chem.* 167, 429-475
- [16] Leshchinskaya, I., Balaban, N., Egorova, G., Taniashin, V., and Tretiak, T. (1974). Isolation and characterization of highly purified preparation of nuclease from *Serratia marcescens*. *Biokhimiya* 39, 116-122
- [17] Miller, M., and Krause, K. (2008). Identification of the *Serratia* endonuclease dimer: structural basis and implications for catalysis. *J. Protein Sci.* 5, 24-33. doi: 10.1002/pro.5560050104
- [18] Miller, M., Tanner, J., Alpaugh, M., Benedik, M., and Krause, K. (1994). 2.1 www.frontiersin.org structure of *Serratia* endonuclease suggests a mechanism for binding to double-stranded DNA. *J. Nat. Struct. Biol.* 1, 461-468. doi: 10.1038/nsb0794-461
- [19] Nestle, M., and Roberts, W. (1969). An extracellular nuclease from *Serratia marcescens*. I. Purification and some properties of the enzyme. *J. Biol. Chem.* 244, 5213-5218
- [20] Pedersen, J., Filimonova, M., Roepstorff, P., and Biedermann, K. (1993). Characterization of *Serratia marcescens* nuclease isoforms by plasma desorption mass spectrometry. *Biochim. Biophys. Acta* 1202, 12-31. doi: 10.1016/0167-4838(93)90057-X
- [21] Romanova, J., and Filimonova, M. (2012). The effects of addition of mononucleotides on *Sma* nuc endonuclease activity. *Sci. World J.* 2012:454176. doi: 10.1100/2012/454176
- [22] Romanova, J., Gubskaya, V., Nuretdinov, I., Zainutdinova, E., and Filimonova, M. (2016). Analysis of the mechanism of Mg^{2+} action on the RNase activity of *Serratia marcescens* endonuclease. *BioNanoScience* 7, 276-283. doi: 10.1007/s12668-016-0358-y
- [23] Shlyapnikov, S., Lunin, V., Perbandt, M., Polyakov, K., Betzel, C., and Mikhailov, A. (2000). Atomic structure of the *Serratia marcescens* endonuclease at 1.1 Å resolution and the enzyme reaction mechanism. *Acta Crystallogr. D Biol. Crystallogr.* 56, 567-572. doi: 10.1107/S090744490000322X
- [24] Trifonova, E., Saveleva, A., Romanova, A., Filipenko, M., Sapotckii, V., Malinovskii, A., et al. (2015). Transgenic expression of *Serratia marcescens* native and mutant nucleases modulates tobacco mosaic virus resistance in *Nicotiana tabacum* L. *Genetika* 51, 835-840. doi: 10.1134/S1022795415070133
- [25] Vafina, G., Bulatov, E., Zainutdinova, E., and Filimonova, M. (2016). A one-step protocol for chromatographic purification of non-recombinant exogenous bacterial enzyme: nuclease of *Serratia marcescens*. *BioNanoScience* 6, 335-337. doi: 10.1007/s12668-016-0226-9
- [26] Voronkova, A., Shmarina, G., Dubovik, L., Kashirskaya, N., and Kapranov, N. (2006). Dornase alfa: clinical and laboratory effects. *Russ. Pulmonol.* 3, 25-28
- [27] Warburg, O., and Christian, W. (1942). Isolation and crystallization of enolase. *Biochem. Z.* 310, 384-421
- [28] Wegrzyn, G., Neubauer, P., Krueger, S., Hecker, M., and Taylor, K. (1991). Stringent control of replication of plasmids derived from coliphage. *Mol. Gen. Genet.* 225, 94-98. doi: 10.1007/BF00282646
- [29] Whitaker, J., and Granum, P. (1980). An absolute method for protein determination based on difference in absorbance at 235 and 280 nm. *Anal. Biochem.* 109, 156-159. doi: 10.1016/0003-2697(80)90024-X